Eur. J. Clin. Chem. Clin. Biochem.
Vol. 30, 1992, pp. 281–284

Neurokinin Concentrations in Cerebrospinal Fluid A Preliminary Study in *Parkinson*'s Disease

By *R. Galard*[1], *R. Catalán*[1], *J. Montalbán*[2], *A. Molins*[2], *F. Miquel*[2] and *J. M. Castellanos*[1]

[1] *Department of Biochemistry*
[2] *Department of Neurology*
Valle Hebrón General Hospital, Barcelona, Spain

(Received March 11, 1991/February 14, 1992)

Summary: Immunoreactive neurokinin A was measured in the cerebrospinal fluid of twelve patients with *Parkinson*'s disease and eleven normal subjects, using a sensitive and precise extraction/concentration radioimmunoassay method. The mean value obtained in *Parkinson*'s disease patients (13.2 ± 4.6 pmol/l) was lower than that of the controls (17.4 ± 5.9). The tendency toward a significant decrease ($p = 0.085$) found in this preliminary study could indicate that neurokinin A-containing neurons are involved in the pathophysiology of *Parkinson*'s disease. In addition, the establishment of reference values for neurokinin A in cerebrospinal fluid may provide a basis for further studies of this neuropeptide in neurological disorders.

Introduction

Neurokinin A and neurokinin B, two decapeptides that belong to the kassinin-like tachykinin family, have been identified as naturally ocurring peptides from mammalian spinal cords (1, 2). Research in the tachykinin field has focussed on clearing up the physiological role of neurokinin A in central and peripheral neural transmission. As predicted from the tachykinin structure of substance P and neurokinin A, it has been reported that these two substances have similar biological effects (3, 4). On the other hand, certain differences in the pharmacological properties of substance P and neurokinin A are basically explained by their interaction with different receptors; the existence of three types of neurokinin receptor is proposed, i. e. NK-1, NK-2 and NK-3, their respective endogenous ligand being substance P, neurokinin A and neurokinin B (5). Furthermore, two different tachykinin mRNAs have been identified in mammals and both of them also encode substance P (6), making tissular coexistence of the two peptides highly probable. In fact, the co-localization of substance P and neurokinin A in several mammalian tissues has already been described (7, 8).

In rats, neurokinin A has been detected across brain regions (9) and some effects on motor behaviour and nociception have been demonstrated (3, 10). It has been suggested that neurokinin A may have a neurotransmitter/neuromodulator role in the substantia nigra of rats (11). Since recent studies have revealed interactions between the dopaminergic system and neurokinin A in the striatonigral pathway (12, 13), we have considered the possibility that neurokinin A levels could be altered in those pathologies where the dopaminergic system is affected.

The aim of this study was to establish reference values of immunorective neurokinin A in human CSF using an extraction/concentration/radioimmunoassay method, and to compare the results with those obtained from patients with *Parkinson*'s disease.

Patients and Methods

Subjects

Lumbar CSF was collected from twelve patients (7 men, 5 women; aged 52 to 72 years) with *Parkinson*'s disease, admitted to the Neurology Department of the Valle Hebron Hospital for a clinical investigation that included spinal puncture. Lumbar

puncture was performed in the morning at bedrest after an overnight fast. Patients had been moderately to severely affected with symptoms for 1 to 11 years. Four were classified as stage I or II, according to *Hoen & Yahr* (14), and eight were included in stages III, IV, and V. Six patients received levodopa combined with extracerebral inhibitor of dopa decarboxylase, five received levodopa plus bromocriptine, and one was taking only bromocriptine. Drug therapy was not stopped. The comparison group included eleven patients from the Emergency Room (aged 50 to 73 years) who had symptoms requiring diagnostic lumbar puncture, but in whom neurological or internal disorders were excluded after careful investigation.

In both groups of patients the biochemical and cytologic CSF analyses were normal and they gave informed consent before participating.

Samples

The CSF samples for immunoreactive neurokinin A assays were centrifuged. Aprotinin (Trasylol®, 1000 · 10^3 KIU/l) was added to prevent enzymatic degradation, and samples were stored at −20 °C until assayed. Blood-tinged CSF was discarded.

Neurokinin A radioimmunoassay

Small columns packed with octadecasilyl silica (C_{18} Sep-pak, Water Assoc., MA, USA) were used for sample extraction as described previously (15). The extracted samples were taken to dryness and resuspended in 500 µl of 0.05 mol/l phosphate buffer (pH = 7.4) containing 2 g/l gelatin and 1 mmol/l EDTA.

Immunoreactive neurokinin A concentrations were measured using a commercially available ^{125}I reagent pak RIA from Amersham laboratories (Code IM 1681) and a neurokinin A standard supplied by Sigma (St Louis, MO, USA). The antiserum provided in this reagent pack showed a 40% cross reaction with neurokinin B and less than 0.1% for substance P. The duplicate RIA tubes containing either 200 µl of known neurokinin A concentration (4.9−79.5 fmol/tube) or 200 µl of dissolved extracted samples were incubated for 24 hours at 4 °C with 100 µl of diluted antiserum (1/10) and 100 µl of labelled neurokinin A (116−100 Bq). The separation of the free from the antibody-bound peptide was performed by adding 100 µl horse serum and 1 ml of polyethylene glycol (20% in distilled water). After centrifugation (1500 *g*, 20 min) the supernatant was decanted and the radioactivity of the precipitate was measured in a gamma counter (LKB-Wallac, 1275 Minigamma with a Databox 1222 calculation unit). Results were plotted as logit versus log concentration of standards. Calculations to determine immunoreactive neurokinin A concentrations (pmol/l) in the CSF samples corresponded to the volume extracted. Sensitivity, defined as the amount of neurokinin A detectable at the 95% confidence level, was 3.5 fmol/tube. Recovery values, obtained by adding 40, 80, and 160 fmol of neurokinin A standard to three different pools, were 95 ± 6%, 99 ± 6% and 97 ± 4% (n = 9). The intra-assay coefficient of variation was studied at two concentrations, CV = 8.6% (x = 3.6 pmol/l; n = 13) and CV = 5.7% (x = 5.8 pmol/l; n = 12). Specificity: different aliquots of three extracted CSF pools, when analysed by the described RIA, gave dose response curves parallel to that of standard neurokinin A (fig. 1). A lineal relationship between expected and found immunoreactive neurokinin A values was observed: y = 0.90x + 0.054, r = 0.9765, P < 0.01 (n = 10).

Statistics

The *Mann-Whitney* U test was employed to determine significant differences between groups. Correlation was established by the *Spearman* correlation coefficient. Data are expressed as mean ± standard deviation.

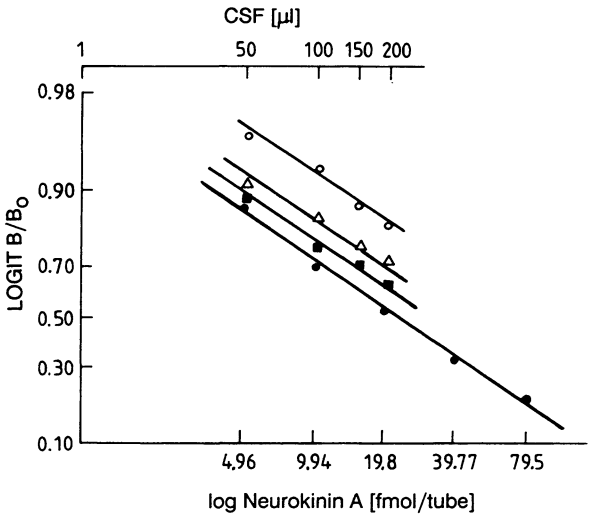

Fig. 1. Effect of increasing aliquots of three extracted CSF pools (○, △, ■) on inhibition (B_0) of maximum binding. The curves obtained were parallel to that of the human neurokinin A standard (●).

Results

The individual values of immunoreactive neurokinin A in CSF of *Parkinson*'s disease patients and controls are shown in figure 2.

The mean value obtained in *Parkinson*'s disease patients (13.2 ± 4.6 pmol/l) was lower than in the controls (17.4 ± 5.9) (mean ± SD). The significance level between the values obtained in the two groups studied was p = 0.085.

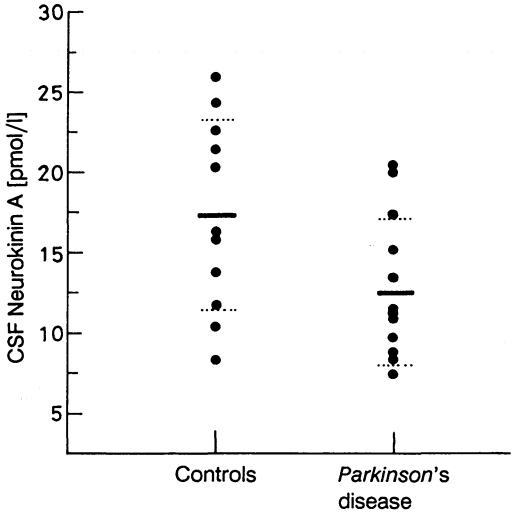

Fig. 2. Individual concentrations of neurokinin A in CSF of control patients (n = 11) and patients with *Parkinson*'s disease (n = 12). Bars indicate mean value. Dashed lines represent standard deviation.

Discussion

To our knowledge, reference values for human CSF immunoreactive neurokinin A have not hitherto been determined. With the RIA method described we have obtained good sensitivity, high recovery values and satisfactory variation coefficients. Also, although our antiserum recognizes neurokinin B, the parallelism of the dilution curves proved that the immunoreactive material determined behaved as the neurokinin A standard. This was expected, since it has been demonstrated, using highly specific antisera, that the concentrations of neurokinin A in several brain regions in mammals are considerably higher than those of neurokinin B (16). Therefore, the CSF tachykinin immunoreactivity measured with our antiserum was mostly due to neurokinin A activity. The establishment of normal neurokinin A CSF values may provide a basis for further studies of this neuropeptide in neurological disorders.

Studies on the regional distribution of tachykinins in the rat central nervous system have shown that neurokinin A is concentrated in areas known to be rich in substance P, such as the midbrain, basal ganglia and spinal cord, the brain region containing the highest concentration of both substance P and neurokinin A being the substantia nigra (9). Also it has been demonstrated that neurokinin A excites the nigral dopaminergic neurons more effectively than substance P, and exhibits a much greater action on locomotor activity (11). The major biochemical characteristic of *Parkinson*'s disease is a degeneration of the dopaminergic neurons of the mesencephalon, although other ascending systems are also affected (17). Significant decreases in substance P have been found in the substantia nigra in *Parkinson*'s disease (18, 19) but similar studies with neurokinin A have not been performed. However, in this preliminary study we found a reduced CSF neurokinin A concentration in twelve *Parkinson*'s disease patients (13.2 $\pm$ 4.6 pmol/l), compared with eleven controls (17.4 $\pm$ 5.9), with a distinct tendency toward significance ($p = 0.085$). The measurement of CSF neurokinin A concentration, in a larger sample of patients, together with brain tissue studies of this neuropeptide in *Parkinson*'s disease, may demonstrate a significant neurokinin A reduction in *Parkinson*ian patients and indicate the involvement of neurokinin A-containing neurons in this disease. Whether the loss of an excitatory neurokinin A input to the substantia nigra contributes to the symptoms of *Parkinson*'s disease may be established in subsequent studies.

Acknowledgement

This work was supported in part by a grant from the Fondo de Investigaciones Sanitarias de la Seguridad Social (91/1154), Ministerio de Educación y Ciencia, Spain.

We wish to thank Mr. *G. Gaspar* and Mrs. *T. Ortiz* for their skilful technical assistance.

References

1. Kanazawa, I., Minamino, N., Fukuda, A. & Matsuo, H. (1980) Neuromedin K: a novel mammalian tachykinin identified in porcine spinal cord. Biochem. Biophys. Res. Commun. *114*, 533–540.
2. Kimura, S., Okada, M., Sugita, Y., Kanazawa, Y. & Munekata, E. (1983) Novel neuropeptides neurokinin A and B, isolated from porcine spinal cord. Proc. Jap. Acad. *53*, 101–104.
3. Hall, M. E., Grantham, P., Limoni, J. & Stewart, J. M. (1987) Effects of substance P and neurokinin A on motor behavior: unique effect of substance P attributable to its amino-terminal sequence. Brain Res. *420*, 82–94.
4. Holzer, U., Lembeck, F. & Seitz, H. (1987) Contractile effects of substance P and neurokinin A on the rat stomach in vivo and in vitro. Br. J. Pharmac. *90*, 273–279.
5. Burcher, E. & Chahl, L. A. (1988) Tools for tachykinin and neuropeptide research. Neurosci. Lett. *86*, 38–44.
6. Krause, J. E., Chirgwin, J. M., Carter, M. S., Xu, Z. S. & Hershey, A. D. (1987) Three rat preprotachykinin mRNAs encode the neuropeptides substance P and neurokinin A. Proc. Natl. Acad. Sci. USA *84*, 881–885.
7. Deacon, C. F., Agoston, D. V. & Conlon, J. M. (1987) Conversion of neuropeptide K to neurokinin A and vesicular colocalization of neurokinin A and substance P in neurons of the guinea pig small intestine. J. Neurochem. *48*, 141–146.
8. Wakisaka, S., Ichikawa, H., Nishiklawa, S., Matsuo, S., Takano, Y. & Akai, M. (1988) Neurokinin like immunoreactivity in feline dental pulp: its distribution, origin and coexistence with substance P-like immunoreactivity. Cell. Tissue Res. *251*, 565–569.
9. Arai, H. & Emson, P. C. (1986) Regional distribution of neuropeptide K and other tachykinins (neurokinin A, neurokinin B and substance P) in rat central nervous system. Brain Res. *399*, 240–249.
10. Laneuville, O., Dorais, J. & Couture, R. (1988) Characterization of the effects produced by neurokinins and three agonists selective for neurokinin receptor subtypes in a spinal nociceptive reflex of the rat. Life Sci. *42*, 1295–1305.
11. Diez Guerra, F. J., Sirinathsinghji, D. J. S. & Emson, P. C. (1988) In vitro and in vivo release of neurokinin A like immunoreactivity from rat substantia nigra. Neuroscience *27*, 527–536.
12. Li, S. J., Sivam, P., McGinty, J. F., Hang, Y. S. & Hong, J. S. (1987) Dopaminergic regulation of tachykinin metabolism in the striatonigral pathway. J. Pharmacol. Exp. Ther. *243*, 792–798.
13. Baruch, P., Artaud, F., Gogeheu, G., Barbeito, L., Glowinski, J. & Cheramy, A. (1988) Substance P and neurokinin A regulate by different mechanism dopamine release from dendrites and nerve terminals of the nigrostriatal dopaminergic neurons. Neuroscience *25*, 889–898.

14. Hehn, M. M. & Yahr, M. D. (1967) Parkinsonism: onset, progression and mortality. Neurology *17*, 427–442.
15. Catalan, R., Galard, R., Molins, A., Sahuquillo, J. & Castellanos, J. M. (1989) C_{18} cartridge extraction method to measure calcitonin in human cerebrospinal fluid. Clin. Chim. Acta *182*, 309–312.
16. Tatesishi, K., Matsuoka, Y. & Hamaoka, T. (1989) Establishment of highly specific radioimmunoassays for neurokinin A and neurokinin B and determination of tissue distribution of these peptides in rat central nervous system. Regulatory Peptides *24*, 245–257.
17. Sourkès, T. L. (1983) Parkinson's disease and other disorders. In: *Basic Neurochemistry* (Siegel, G. J., Albes, R. W., Agranof, B. W. & Katzman, R., eds.) pp. 719–733, Little Brown, Boston.
18. Mauborgne, A., Javoy-Agid, F. & Legrand, J. C. (1983) Decrease of substance P – like immunoreactivity in the substantia nigra and pallidum of parkinsonian brain. Brain Res. *268*, 167–170.
19. Tenovuo, O., Rinne, U. K. & Viljanen, M. K. (1984) Substance P immunoreactivity in the post-mortem parkinsonian brain. Brain Res. *303*, 113–116.

Dr. R. Galard
Hormone Laboratories
Department of Biochemistry
Valle Hebrón General Hospital
E-08035 Barcelona